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Appeal brief
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S): Oscar J. Llorin et al.

SERIAL NO.: 09/128,340 GROUP: 1651

FILING DATE: August 3, 1998 EXAMINER: D. Ware

FOR: CELL DISRUPTION METHOD USING SONICATION

no fees

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: COMMISSIONER OF PATENTS AND TRADEMARKS, WASHINGTON, D.C. 20231

ON: June 27, 2000 (DATE OF DEPOSIT)

BY: Mary Lou Kittren (NAME)

MaryLou Kittren 6-27-00 (SIGNATURE) (DATE)

Assistant Commissioner for Patents
Washington, D.C. 20231

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APPEAL BRIEF

This is an Appeal from the Final Rejection mailed on January 4, 2000.

37 CFR §1.192(c)(1) -- REAL PARTY IN INTEREST

The real party in interest to this Appeal is Becton, Dickinson and Company, the owner and assignee of the captioned application. The assignment is recorded at Reel/Frame 9372/0140 in the United States Patent and Trademark Office, Assignment Branch.

37 CFR §1.192(c)(2) -- RELATED APPEALS AND INTERFERENCES

The Appellants, the Appellants' legal representative, and the assignee are not aware of other related appeals or interferences that will directly affect, be directly affected by, or have a bearing on the Board's decision in the pending Appeal.

37 CFR §1.192(c)(3) -- STATUS OF THE CLAIMS

Claims 1 and 3-13 are pending.

Claims 1 and 3-13 stand rejected.

Claims 1 and 3-13 are appealed.

37 CFR §1.192(c)(4) -- STATUS OF AMENDMENTS

In response to the Final Rejection, Appellants filed an amendment to claims 5 and 8 under 35 USC §1.116 on March 6, 2000. In an Advisory Action mailed on April 10, 2000, the Examiner indicated that the amendments would be entered upon the filing of an appeal. Claims 1 and 3-13, as pending, appear in Appendix 1, attached hereto.

37 CFR §1.192(c)(5) -- SUMMARY OF INVENTION

Broadly, the invention is a method for disrupting cells in a sample. In one embodiment of the invention, the cells are in a liquid of alkaline pH, and subjected to ultrasonic energy in the absence of beads. In another embodiment of the invention, the cells are in a liquid of reduced surface tension, and subjected to ultrasonic energy.

37 CFR §1.192(c)(6) -- ISSUES

A. Whether claims 5-13 are properly rejected under 35 USC §112, second paragraph.

B. Whether, under 35 U.S.C. §103(a), the inventions of claims 1 and 3-13 would have been obvious to one of ordinary skill in the art at the time of filing of the present application in view of the disclosure of Buck *et al.* taken in combination with the disclosures of Robson *et al.* and Robbins *et al.*

37 CFR §1.192(c)(7) -- GROUPING OF CLAIMS

Claim 1 is directed to a method for disrupting cells in a liquid of alkaline pH by subjecting the cells to ultrasonic energy in a sonic bath in the absence of beads, and stands rejected as obvious. Claim 1 stands or falls alone.

Claim 3 is directed to a method for disrupting cells in a liquid of alkaline pH by subjecting the cells to ultrasonic energy in a sonic bath in the absence of beads at a temperature of about 65°C to about 75°C, and stands rejected as obvious. Claim 3 stands or falls alone.

Claim 4 is directed to a method for disrupting mycobacterial cells in a liquid of alkaline pH, by subjecting the mycobacterial cells to ultrasonic energy in a sonic bath in the absence of beads at a temperature of about 65°C to about 75°C, and stands rejected as obvious. Claim 4 stands or falls alone.

Claim 5 is directed to a method for disrupting cells in a liquid of alkaline pH and reduced surface tension by subjecting the cells to ultrasonic energy in a sonic bath in the absence of beads, and stands rejected as indefinite and obvious. Claim 5 stands or falls alone.

Claim 6 is directed to a method for disrupting cells in a liquid of alkaline pH and reduced surface tension by subjecting the cells to ultrasonic energy in a sonic bath in the absence of beads at a temperature of about 65°C to about 75°C, and stands rejected as indefinite and obvious. Claim 6 stands or falls alone.

Claim 7 is directed to a method for disrupting mycobacterial cells in a liquid of alkaline pH and reduced surface tension by subjecting the mycobacterial cells to ultrasonic energy in a sonic bath in the absence of beads at a temperature of about 65°C to about 75°C, and stands rejected as indefinite and obvious. Claim 7 stands or falls alone.

Claim 8 is directed to a method for disrupting cells in a liquid of reduced surface tension by subjecting the cells to ultrasonic energy, and stands rejected as indefinite and obvious. Claim 8 stands or falls alone.

Claim 9 is directed to a method for disrupting cells in a liquid of reduced surface tension by subjecting the cells to ultrasonic energy in a sonic bath, and stands rejected as indefinite and obvious. Claim 9 stands or falls alone.

Claim 10 is directed to a method for disrupting cells in a liquid of reduced surface tension by subjecting the cells to ultrasonic energy in the presence of beads, and stands rejected as indefinite and obvious. Claim 10 stands or falls alone.

Claim 11 is directed to a method for disrupting cells in a liquid of reduced surface tension and alkaline pH by subjecting the cells to ultrasonic energy, and stands rejected as indefinite and obvious. Claim 11 stands or falls alone.

Claim 12 is directed to a method for disrupting cells in a liquid of reduced surface tension by subjecting the cells to ultrasonic energy in a sonic bath at a temperature of about 65°C to about 75°C, and stands rejected as indefinite and obvious. Claim 12 stands or falls alone.

Claim 13 is directed to a method for disrupting mycobacterial cells in a liquid of reduced surface tension by subjecting the mycobacterial cells to ultrasonic energy in a sonic bath at a temperature of about 65°C to about 75°C, and stands rejected as indefinite and obvious. Claim 13 stands or falls alone.

37 CFR §1.192(c)(8) -- ARGUMENTS

I. Indefiniteness (35 U.S.C. §112, Second Paragraph)

Claims 5-13 stand rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Advisory Action mailed on April 10, 2000 stated that claims 5-13 remain rejected under 35 U.S.C. §112, second paragraph for those reasons of record. The reasons of record set forth in the Final Rejection of January 4, 2000 were that:

> [c]laims 5-13 are rendered indefinite for the recitation of "the standard state surface tension of said second liquid is reduced" and "the standard state surface

tension of said first liquid is reduced" at lines 1-3 and 2-3, recited respectively in claims 5 and 8. The recitations in these claims lack antecedent basis. Dependent claims 6-7 and 9-13 are rejected for being dependent upon a rejected base claim.

A. Claim 5

As explained in the Amendment filed on March 6, 2000, claim 5 was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 5 now calls for the reduction of the surface tension of a particular liquid. An inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 5 because claim 1, from which claim 5 depends, recites a second liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property within a dependent claim. Thus, claim 5 meets the requirements of 35 U.S.C. §112, second paragraph.

B. Claim 6

Claim 6 depends from claim 5 that was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 6 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 6 because claim 1, from which claim 5 depends, recites a second liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property within a dependent claim. Thus, claim 6 meets the requirements of 35 U.S.C. §112, second paragraph.

C. Claim 7

Claim 7 depends from claim 6, and claim 6 depends from claim 5 that was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 7 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 7 because claim

1, from which claim 5 depends, recites a second liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property in a dependent claim. Thus, claim 7 meets the requirements of 35 U.S.C. §112, second paragraph.

D. Claim 8

As explained in the Amendment filed on March 6, 2000, claim 8 was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 8 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 8 because that claim recites a first liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property within the same claim. Thus, claim 8 meets the requirements of 35 U.S.C. §112, second paragraph.

E. Claim 9

Claim 9 depends from claim 8 that was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 9 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 9 because claim 8 recites a first liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property within a dependent claim. Thus, claim 9 meets the requirements of 35 U.S.C. §112, second paragraph.

F. Claim 10

Claim 10 depends from claim 8 that was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 10 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus

proper antecedent basis is present for claim 10 because claim 8 recites a first liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property within a dependent claim. Thus, claim 10 meets the requirements of 35 U.S.C. §112, second paragraph.

G. Claim 11

Claim 11 depends from claim 8 that was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 11 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 11 because claim 8 recites a first liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property within a dependent claim. Thus, claim 11 meets the requirements of 35 U.S.C. §112, second paragraph.

H. Claim 12

Claim 12 depends from claim 9, and claim 9 depends from claim 8 that was amended to address this rejection by deletion of the phrase "standard state." Thus, claim 12 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 12 because claim 8, from which claims 9 and 12 depend, recites a first liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property in a dependent claim. Thus, claim 12 meets the requirements of 35 U.S.C. §112, second paragraph.

I. Claim 13

Claim 13 depends from claim 12, and claim 12 depends from claim 9, and claim 9 depends from claim 8 which was amended to address this rejection by deletion of the phrase

"standard state." Thus, claim 13 now calls for the reduction of the surface tension of a particular liquid. As stated above, an inherent property of a liquid is its surface tension, and thus proper antecedent basis is present for claim 13 because claim 8, from which claims 9, 12 and 13 depend, recites a first liquid. It is not necessary to recite an inherent property of a component of a claimed invention in order to reference a change in such a property in a dependent claim. Thus, claim 13 meets the requirements of 35 U.S.C. §112, second paragraph.

II. Obviousness (35 U.S.C. §103(a))

Claims 1 and 3-13 stand rejected under 35 U.S.C. §103 (a) as being unpatentable over Buck *et al.* in view of Robson *et al.* and Robbins *et al.* Through the Office Actions for the present application, it has been specifically asserted that:

- Buck teaches sonication of mycobacteria in a liquid having an alkaline pH (Office Action mailed on March 30, 1999, Office Action mailed on August 30 1999 and Office Action mailed on January 4, 2000);
- Buck teaches that sonication and the usage of an alkalinized solution for treating cells for purposes of disrupting them are well known techniques in the art (Office Action mailed on January 4, 2000);
- Robson teaches lysis or disintegration of mycobacteria using liquids having an alkaline pH, and teaches a sonication procedure on mycobacteria (Office Action mailed on August 30, 1999 and Office Action mailed on January 4, 2000);
- Robbins teaches cells contained in a liquid having an alkaline pH, as well as sonic disintegration of the cells to obtain an alkaline extract therefrom (Office Action mailed on August 30, 1999 and Office Action mailed on January 4, 2000);

- Robbins teaches the application of an alkaline pH for preparation of cells before sonication to cause disruption of the cells (Office Action mailed on January 4, 2000).

A. Claim 1

1. The assertions regarding Buck are incorrect

Buck does not teach sonication of mycobacteria in a liquid having an alkaline pH as asserted in the Office Actions mailed on March 30, 1999, August 30, 1999 and January 4, 2000. The sonication method of Buck ("method iv") is not conducted with a liquid having an alkaline pH.

The assertions in the Office Actions attempt to tie the PCR buffer (pH 8.3) in method iii to the liquid used for sonication in method iv. However, method iv only refers to method iii for a centrifugation technique. Specifically, the description of method iv states that "[t]he suspensions were centrifuged as described above for method iii, ..." The description of method iii states that "[s]uspensions were centrifuged at 16,000 x g for 5 min, and the fluid poured off."

At this point method iv and method iii diverge. In method iv, the centrifuged suspensions are "washed twice with distilled water, and then resuspended in the residual water (approximately 25 ul)" (emphasis added). In contrast, in method iii, "[f]ifty microliters of either distilled water or 2% Triton X-100 in PCR buffer" is added to the centrifuged suspensions. In its sonication method, Buck teaches only the use of distilled water, and there is no suggestion that PCR buffer would be a suitable alternative to water.

Furthermore, Buck fails to teach one skilled in the art of the enhancement of a sonication cell disruption method by alkalization of a sonication solution. The use of PCR buffer in methods i, ii and iii of Buck are not critical to cell disruption. Rather, the use of PCR buffer in each of these methods serves only to secure optimal pH conditions for *Taq* DNA polymerase enzyme.

2. There are significant differences between the teachings of Buck and the claimed invention

The manner of sonication described by Buck is fundamentally different from the method of claim 1. Specifically, the method of claim 1 uses a sonic bath. In contrast, Buck teaches that "tubes were placed in a plastic rack that was floated in a dish of water next to the sonicator probe (Sonics & Materials, Danbury, Conn.) and sonicated at 45 W" (paragraph bridging pages 1331 and 1332). The disadvantages and disincentives of using a sonicator probe are discussed in the present application at page 2, lines 9-25. These disadvantages and disincentives are further evidenced by Murphy et al. (U.S. 5,374,522) which describes the significant differences between a probe sonic oscillator (sonic probe of Buck) and a sonic bath (see column 9, line 48-column 10, line 12). Due to these significant differences, one of ordinary skill in the art would not be led to use a sonic bath without beads for the lysis of cells from the teaching of Buck which describes a significantly different and inferior method using a sonic probe.

3. The teachings of Robson fail to adequately address the significant differences between the teachings of Buck and the claimed invention

Robson does not provide one of ordinary skill in the art with a teaching sufficient to overcome the deficiencies of Buck. As asserted in the Office Actions mailed on August 30, 1999 and January 4, 2000, Robson teaches the use of liquids having an alkaline pH in mycobaterial lysis methods at column 6, lines 26-40, and teaches a sonication method in Example 4. However, the reference to alkaline pH liquids at column 6 is a mere laundry list of acceptable milieus in which mycobacterial cells may be heated to obtain readily useable components, *i.e.* the invention of the patent. Example 4 is a comparative example in which the sample of mycobacterial cells from a BACTEC system "was reconstituted in 0.5 ml of H_2O plus ~25 µl worth of glass beads" (column 8, lines 57-58).

Not only does Robson fail to teach the use of a liquid having an alkaline pH in comparative Example 4, but has the same failure in all other Examples disclosing sonication. Specifically, in comparative Examples 2 and 5, Robson discloses sonication methods, but as with

comparative Example 4, these Examples teach the reconstitution of a sample of mycobacterial cells from a BACTEC system in water (column 8, lines 1-2 for Example 2; and column 9, lines 16-19 for Example 5). Thus, Robson, like Buck, fails to disclose use of a liquid of alkaline pH in a sonication method for the disruption of cells.

Also, comparative Example 4 of Robson teaches the use of sonication with glass beads, as contrasted with the method of claim 1 wherein sonication is conducted in the absence of beads. Finally, the results of comparative Examples 2, 4 and 5 of Robson were characterized as follows.

-- Example 2 "The autoradiogram of the blot showed that no DNA hybridized to the radioactive probe and therefore sonication treatment alone released no DNA from the *M. tuberculosis*" (column 8, lines 16-19).

-- Example 4 "The autoradiogram of the blot showed that no DNA hybridized to the radioactive probe and therefore sonication plus glass beads did not release enough DNA to be detected or the DNA remained bound to the beads" (column 9, lines 6-9).

-- Example 5 "While Gen-Probe [the lysing tube of Example 5] was successful, two extra phenol/chloroform extractions were required to clear the sample (*i.e.* remove contaminants from the lysis solution) before it was subjected to analysis" (column 9, lines 38-41).

Thus, the best result of all the sonication procedures taught by Robson is a contaminated (*i.e.* dirty) sample. Such results do not constitute a teaching that can be characterized as providing those skilled in the art with a reasonable expectation of success for achieving the claimed invention.

4. The teachings of Robbins fail to adequately address the significant differences between the teachings of Buck and the claimed invention

Robbins also fails to provide one of ordinary skill in the art with a teaching sufficient to overcome the deficiencies of Buck and Robson. In the Office Action mailed on January 4, 2000, column 3, lines 19-23 were cited as providing a teaching of the application of an alkaline pH for preparation of the cells before sonication to cause disruption of the cells. However, as with Buck and Robson, Robbins does not teach one skilled in the art that alkalization of a solution aids cell disruption using sonication. Column 3, lines 19-23 states that "dilute alkali may be incorporated in the wash to remove adhering color and taste bodies" (emphasis added). A wash solution is not the liquid in which sonication takes place. There is no teaching of the nature or pH of a sonication solution in Robbins. Further, to the extent that any pH disclosure of Robbins may be arguably relevant, it would be the pH used with the preferred homogenization cell rupture method which is performed at an acidic pH of 4.5-6.5 (column 3, line 32). Robbins disclosure of pH adjustment at column 3, lines 32-35 relates to a post-homogenization ruptured yeast cell system, not to the solution used during the cell rupture process.

5. Conclusion – There are significant differences between the combination of cited references and the claimed invention, and a lack of suggestion or motivation to combine the cited references

There are significant differences between the invention of claim 1 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to alkalize a sonication solution to enhance cell disruption is lacking, because, to the extent the cited references utilize alkaline solutions, there is no recognition of its utility in a sonication based cell disruption method. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 1.

B. Claim 3

Claim 3 depends from claim 1, and thus all of the arguments above for claim 1 are equally applicable for claim 3. In addition, claim 3 calls for the temperature of the liquid of the sonic bath to be about 65°C to about 75°C. Method iv of Buck (sonication method) does not disclose a temperature for the dish of water next to the sonicator probe, and thus one skilled in the art would presume this temperature to be room temperature at the onset of sonication. Because the distances between the tubes in the dish of water and the probe of Buck are not disclosed, Buck does not provide one of ordinary skill in the art with sufficient guidance regarding the temperature of the dish of water next to the sonication probe.

Robson does not cure this deficiency of Buck, because Robson discloses sonication at 60°C in Examples 2, 4 and 5 (the only sonication examples of Robson). Similarly, Robbins fails to cure this deficiency of Buck and Robson, because Robbins, like Buck, fails to disclose a temperature for sonic disintegration. To the extent that Robbins discloses an arguably relevant temperature, it is the temperature at which the preferred homogenization method of cell rupture is conducted, namely 32°F to 122°F (column 3, lines 31-32). This temperature range is 0°C to 50°C. Robbins disclosure of warming at column 3, lines 32-35 relates to a post-homogenization ruptured yeast cell system, not to the solution used during the cell rupture process.

Thus, there are significant differences between the invention of claim 3 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to alkalize a sonication solution to enhance cell disruption is lacking, because, to the extent the cited references utilize alkaline solutions, there is no recognition of its utility in a sonication based cell disruption method. Similarly, the cited references fail to teach temperatures for sonication in the range specified in claim 3. One skilled

in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 3.

C. Claim 4

Claim 4 depends from claim 3, which depends from claim 1, and thus all of the arguments above for claims 1 and 3 are equally applicable for claim 4. In addition, claim 4 requires that the cells subjected to the claimed method be mycobacterial cells. Although Buck and Robson disclose methods to lyse mycobacterial cells, Robbins teaches the rupture of yeast cells.

Thus, there are significant differences between the invention of claim 4 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to alkalize a sonication solution to enhance cell disruption is lacking, because, to the extent the cited references utilize alkaline solutions, there is no recognition of its utility in a sonication based cell disruption method. Similarly, the cited references fail to teach temperatures for sonication in the range specified in claim 4. Also, Robbins fails to teach the disruption of mycobacterial cells. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 4.

D. Claim 5

Claim 5 depends from claim 1, and thus all of the arguments above for claim 1 are equally applicable for claim 5. In addition, claim 5 calls for the surface tension of the liquid in which the cells are present to be reduced. The sonication methods disclosed in Buck and Robson fail to teach any reduction of the surface tension of the liquid in which the cells are present

during sonication. Similarly, Robbins, in its homogenization cell rupture method, fails to teach a reduction of the surface tension of any liquid.

Thus, there are significant differences between the invention of claim 5 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to alkalize a sonication solution to enhance cell disruption is lacking, because, to the extent the cited references utilize alkaline solutions, there is no recognition of its utility in a sonication based cell disruption method. Similarly, the cited references fail to teach a reduction of the surface tension of the liquid in which the cells are present as specified in claim 5. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 5.

E. Claim 6

Claim 6 depends from claim 5, which depends from claim 1, and thus all of the arguments above for claims 1 and 5 are equally applicable for claim 6. In addition, claim 6 calls for the temperature of the liquid of the sonic bath to be about 65°C to about 75°C. Method iv of Buck (sonication method) does not disclose a temperature for the dish of water next to the sonicator probe, and thus one skilled in the art would presume this temperature to be room temperature at the onset of sonication. Because the distances between the tubes in the dish of water and the probe of Buck are not disclosed, Buck does not provide one of ordinary skill in the art with sufficient guidance regarding the temperature of the dish of water next to the sonication probe.

Robson does not cure this deficiency of Buck, because Robson discloses sonication at 60°C in Examples 2, 4 and 5 (the only sonication examples of Robson). Similarly, Robbins fails to cure this deficiency of Buck and Robson, because Robbins, like Buck, fails to disclose a temperature for sonic disintegration. To the extent that Robbins discloses an arguably relevant

temperature, it is the temperature at which the preferred homogenization method of cell rupture is conducted, namely 32°F to 122°F (column 3, lines 31-32). This temperature range is 0°C to 50°C. Robbins disclosure of warming at column 3, lines 32-35 relates to a post-homogenization ruptured yeast cell system, not to the solution used during the cell rupture process.

Thus, there are significant differences between the invention of claim 6 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to alkalize a sonication solution to enhance cell disruption is lacking, because, to the extent the cited references utilize alkaline solutions, there is no recognition of its utility in a sonication based cell disruption method. Similarly, the cited references fail to teach reduction of the surface tension of the liquid in which the cells are present and the temperatures for sonication in the range specified in claim 6. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 6.

F. Claim 7

Claim 7 depends from claim 6, which depends from claim 5, which depends from claim 1, and thus all of the arguments above for claims 1, 5 and 6 are equally applicable for claim 7. In addition, claim 7 requires that the cells subjected to the claimed method be mycobacterial cells. Although Buck and Robson disclose methods to lyse mycobacterial cells, Robbins teaches the rupture of yeast cells.

Thus, there are significant differences between the invention of claim 7 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to alkalize a sonication solution to enhance cell disruption is lacking, because, to the extent the cited references utilize alkaline solutions, there is no recognition of its utility in a sonication based cell disruption method. Similarly, the cited references fail to teach reduction of the surface tension of the liquid in which the cells are present and the temperatures for sonication in the range specified in claim 7. Also, Robbins fails to teach the disruption of mycobacterial cells. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 7.

G. Claim 8

1. There are significant differences between the teachings of Buck and the claimed invention

Buck does not teach sonication of mycobacteria in a liquid having a reduced surface tension. The sonication method of Buck ("method iv") is not conducted with a liquid having a reduced surface tension, and Buck fails to teach one skilled in the art of the enhancement of a sonication cell disruption method by reducing the surface tension of a sonication solution.

2. The teachings of Robson fail to adequately address the significant differences between the teachings of Buck and the claimed invention

Robson does not provide one of ordinary skill in the art with a teaching sufficient to overcome the deficiencies of Buck. Robson also fails to teach a reduction of the surface tension of a sonication solution. Thus, Robson, like Buck, fails to disclose use of a liquid of reduced surface tension in a sonication method for the disruption of cells. Furthermore, the results of the Examples in which Robson conducts a sonication method (comparative Examples 2, 4 and 5) were characterized as follows.

-- Example 2 "The autoradiogram of the blot showed that no DNA hybridized to the radioactive probe and therefore sonication treatment alone released no DNA from the *M. tuberculosis*" (column 8, lines 16-19).

-- Example 4 "The autoradiogram of the blot showed that no DNA hybridized to the radioactive probe and therefore sonication plus glass beads did not release enough DNA to be detected or the DNA remained bound to the beads" (column 9, lines 6-9).

-- Example 5 "While Gen-Probe [the lysing tube of Example 5] was successful, two extra phenol/chloroform extractions were required to clear the sample (*i.e.* remove contaminants from the lysis solution) before it was subjected to analysis" (column 9, lines 38-41).

Thus, the best result of all the sonication procedures taught by Robson is a contaminated (*i.e.* dirty) sample. Such results do not constitute a teaching that can be characterized as providing those skilled in the art with a reasonable expectation of success for achieving the claimed invention.

3. The teachings of Robbins fail to adequately address the significant differences between the teachings of Buck and the claimed invention

Robbins also fails to provide one of ordinary skill in the art with a teaching sufficient to overcome the deficiencies of Buck and Robson, because Robbins fails to teach the reduction of the surface tension of a sonication solution.

4. Conclusion – There are significant differences between the combination of cited references and the claimed invention, and a lack of suggestion or motivation to combine the cited references

There are significant differences between the invention of claim 8 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to reduce the surface tension of a sonication solution to enhance cell disruption is lacking, because, the cited references fail to recognize its utility in a sonication based cell disruption method. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 8.

H. Claim 9

Claim 9 depends from claim 8, and thus all of the arguments above for claim 8 are equally applicable to claim 9. In addition, claim 9 calls for the use of a vessel in a sonic bath.

The manner of sonication described by Buck is fundamentally different from the method of claim 9. Specifically, the method of claim 9 uses a sonic bath. In contrast, Buck teaches that "tubes were placed in a plastic rack that was floated in a dish of water next to the sonicator probe (Sonics & Materials, Danbury, Conn.) and sonicated at 45 W" (paragraph bridging pages 1331 and 1332). The disadvantages and disincentives of using a sonicator probe are discussed in the present application at page 2, lines 9-25. These disadvantages and disincentives are further evidenced by Murphy et al. (U.S. 5,374,522) which describes the significant differences between a probe sonic oscillator (sonic probe of Buck) and a sonic bath (see column 9, line 48-column 10, line 12). Due to these significant differences, one of ordinary skill in the art would not be led to use a sonic bath for the lysis of cells from the teaching of Buck which describes a significantly different and inferior method using a sonic probe.

Robson does not provide one of ordinary skill in the art with a teaching sufficient to overcome the deficiencies of Buck. Robson fails to specify whether a sonic bath is used in its sonication methods. Similarly, Robbins fails to specify the use of a sonic bath for its sonic disintegration cell rupture method.

Thus, there are significant differences between the invention of claim 9 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient

suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to reduce the surface tension of a sonication solution to enhance cell disruption is lacking, because, the cited references fail to recognize its utility in a sonication based cell disruption method. Similarly, the cited references fail to provide adequate motivation for one skilled in the art to utilize beads in such a cell disruption method. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 9.

I. Claim 10

Claim 10 depends from claim 8, and thus all of the arguments above for claim 8 are equally applicable to claim 10. In addition, claim 10 calls for the presence of beads in the liquid in which the cells are present.

Buck and Robbins fail to teach the use of beads in their respective disclosed sonication methods. Robson discloses the use of beads, but the results achieved by Robson, as summarized above, would not provide those skilled in the art with a reasonable expectation of success.

Thus, there are significant differences between the invention of claim 10 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to reduce the surface tension of a sonication solution to enhance cell disruption is lacking, because, the cited references fail to recognize its utility in a sonication based cell disruption method. Similarly, the cited references fail to provide adequate motivation for one skilled in the art to utilize a sonic bath in such a cell disruption method. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 10.

J. Claim 11

Claim 11 depends from claim 8, and thus all of the arguments above for claim 8 are equally applicable to claim 11. In addition, claim 11 calls for the liquid in which the cells are present to be of an alkaline pH.

Buck does not teach sonication of cells in a liquid having an alkaline pH. The sonication method disclosed by Buck ("method iv") is not conducted with water rather than a liquid having an alkaline pH.

The assertions in the Office Actions attempt to tie the PCR buffer (pH 8.3) in method iii to the liquid used for sonication in method iv. However, method iv only refers to method iii for a centrifugation technique. Specifically, the description of method iv states that "[t]he suspensions were centrifuged as described above for method iii, ..." The description of method iii states that "[s]uspensions were centrifuged at 16,000 x g for 5 min, and the fluid poured off."

At this point method iv and method iii diverge. In method iv, the centrifuged suspensions are "washed twice with distilled water, and then resuspended in the residual water (approximately 25 ul)" (emphasis added). In contrast, in method iii, "[f]ifty microliters of either distilled water or 2% Triton X-100 in PCR buffer" is added to the centrifuged suspensions. In its sonication method, Buck teaches only the use of distilled water, and there is no suggestion that PCR buffer would be a suitable alternative to water.

Furthermore, Buck fails to teach one skilled in the art of the enhancement of a sonication cell disruption method by alkalization of a sonication solution. The use of PCR buffer in methods i, ii and iii of Buck are not critical to cell disruption. Rather, the use of PCR buffer in each of these methods serves only to secure optimal pH conditions for *Taq* DNA polymerase enzyme.

Robson does not provide one of ordinary skill in the art with a teaching sufficient to overcome the deficiencies of Buck. As asserted in the Office Actions mailed on August 30, 1999 and January 4, 2000, Robson teaches the use of liquids having an alkaline pH in mycobaterial lysis methods at column 6, lines 26-40, and teaches a sonication method in Example 4.

However, the reference to alkaline pH liquids at column 6 is a mere laundry list of acceptable milieus in which mycobacterial cells may be heated to obtain readily useable components, *i.e.* the invention of the patent. Example 4 is a comparative example in which the sample of mycobacterial cells from a BACTEC system "was reconstituted in 0.5 ml of H_2O plus ~25 µl worth of glass beads" (column 8, lines 57-58).

Not only does Robson fail to teach the use of a liquid having an alkaline pH in comparative Example 4, but has the same failure in all other Examples disclosing sonication. Specifically, in comparative Examples 2 and 5, Robson discloses sonication methods, but as with comparative Example 4, these Examples teach the reconstitution of a sample of mycobacterial cells from a BACTEC system in water (column 8, lines 1-2 for Example 2; and column 9, lines 16-19 for Example 5). Thus, Robson, like Buck, fails to disclose use of a liquid of alkaline pH in a sonication method for the disruption of cells.

Also, the results of comparative Examples 2, 4 and 5 of Robson were characterized as follows.

-- Example 2 "The autoradiogram of the blot showed that no DNA hybridized to the radioactive probe and therefore sonication treatment alone released no DNA from the *M. tuberculosis*" (column 8, lines 16-19).

-- Example 4 "The autoradiogram of the blot showed that no DNA hybridized to the radioactive probe and therefore sonication plus glass beads did not release enough DNA to be detected or the DNA remained bound to the beads" (column 9, lines 6-9).

-- Example 5 "While Gen-Probe [the lysing tube of Example 5] was successful, two extra phenol/chloroform extractions were required to clear

the sample (*i.e.* remove contaminants from the lysis solution) before it was subjected to analysis" (column 9, lines 38-41).

Thus, the best result of all the sonication procedures taught by Robson is a contaminated (*i.e.* dirty) sample. Such results do not constitute a teaching that can be characterized as providing those skilled in the art with a reasonable expectation of success for achieving the claimed invention.

Robbins also fails to provide one of ordinary skill in the art with a teaching sufficient to overcome the deficiencies of Buck and Robson. In the Office Action mailed on January 4, 2000, column 3, lines 19-23 were cited as providing a teaching of the application of an alkaline pH for preparation of the cells before sonication to cause disruption of the cells. However, as with Buck and Robson, Robbins does not teach one skilled in the art that alkalization of a solution aids cell disruption using sonication. Column 3, lines 19-23 states that "dilute alkali may be incorporated in the wash to remove adhering color and taste bodies" (emphasis added). A wash solution is not the liquid in which sonication takes place. There is no teaching of the nature or pH of the sonication solution in Robbins. Further, to the extent that any pH disclosure of Robbins may be arguably relevant, it would be the pH used with the preferred homogenization cell rupture method which is performed at an acidic pH of 4.5-6.5 (column 3, line 32). Robbins disclosure of pH adjustment at column 3, lines 32-35 relates to a post-homogenization ruptured yeast cell system, not to the solution used during the cell rupture process.

Thus, there are significant differences between the invention of claim 11 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to reduce the surface tension of a sonication solution, or to alkalize a sonication solution to enhance cell disruption is lacking, because the cited references fail to teach such a surface tension reduction, and, to the extent the cited

references utilize alkaline solutions, there is no recognition of its utility in a sonication based cell disruption method. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 11.

K. Claim 12

Claim 12 depends from claim 9, which depends from claim 8, and thus all of the arguments above for claims 8 and 9 are equally applicable to claim 12. In addition, claim 12 calls for the temperature of the liquid of the sonic bath to be about 65°C to about 75°C. Method iv of Buck (sonication method) does not disclose a temperature for the dish of water next to the sonicator probe, and thus one skilled in the art would presume this temperature to be room temperature at the onset of sonication. Because the distances between the tubes in the dish of water and the probe of Buck are not disclosed, Buck does not provide one of ordinary skill in the art with sufficient guidance regarding the temperature of the dish of water next to the sonication probe.

Robson does not cure this deficiency of Buck, because Robson discloses sonication at 60°C in Examples 2, 4 and 5 (the only sonication examples of Robson). Similarly, Robbins fails to cure this deficiency of Buck and Robson, because Robbins, like Buck, fails to disclose a temperature for sonic disintegration. To the extent that Robbins discloses an arguably relevant temperature, it is the temperature at which the preferred homogenization method of cell rupture is conducted, namely 32°F to 122°F (column 3, lines 31-32). This temperature range is 0°C to 50°C. Robbins disclosure of warming at column 3, lines 32-35 relates to a post-homogenization ruptured yeast cell system, not to the solution used during the cell rupture process.

Thus, there are significant differences between the invention of claim 12 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the suggestion or motivation to reduce the surface tension of a sonication solution to enhance cell disruption is lacking, because, the cited references do not provide any such teaching, and do not provide any recognition of its utility in a sonication based cell disruption method. Similarly, the cited references fail to specifically disclose use of a sonic bath and fail to teach temperatures for sonication in the range specified in claim 12. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 12.

L. Claim 13

Claim 13 depends from claim 12, which depends from claim 9, which depends from claim 8, and thus all of the arguments above for claims 8, 9 and 12 are equally applicable to claim 13. In addition, claim 13 requires that the cells subjected to the claimed method be mycobacterial cells. Although Buck and Robson disclose methods to lyse mycobacterial cells, Robbins teaches the rupture of yeast cells.

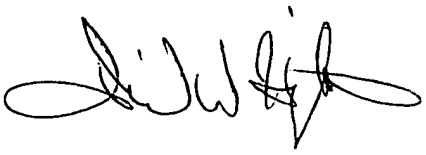
Thus, there are significant differences between the invention of claim 13 and the teachings of the cited references. Further, the teachings of the cited references fail to provide sufficient suggestion or motivation for those skilled in the art to address these significant differences to arrive at the claimed invention with a reasonable expectation of success.

Specifically, the cited references fail to teach reduction of the surface tension of the liquid in which the cells are present and the temperatures for sonication in the range specified. Similarly, the use of a sonic bath as claimed is not specifically disclosed by the cited references. Also, Robbins fails to teach the disruption of mycobacterial cells. One skilled in the art would not be motivated by the cited references to combine their fragmented teachings to yield the invention of claim 13.

CONCLUSION

In view of the foregoing, Appellants respectively assert that the Examiner's rejections cannot be sustained. Reversal of these rejections is therefore, respectfully requested.

Respectfully submitted,



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37 CFR §1.192(c)(9) -- APPENDIX

--1 (twice amended). A method for disrupting cells comprising:

providing a sonic bath comprising a first liquid;

placing into said first liquid a vessel comprising cells in a second liquid at an alkaline pH; and

subjecting said cells to ultrasonic energy from said sonic bath of sufficient power and duration to cause disruption of said cells in the absence of beads.--

--3 (amended). The method of claim 1 wherein the temperature of said first liquid is about 65°C to about 75°C.--

4. The method of claim 3 wherein the cells are mycobacterial cells.

--5 (twice amended). The method of claim 1 wherein the surface tension of said second liquid is reduced.--

6. The method of claim 5 wherein the temperature of said first liquid is about 65°C to about 75°C.

7. The method of claim 6 wherein the cells are mycobacterial cells.

--8 (twice amended). A method for disrupting cells by applying ultrasonic energy to a sample of cells in a first liquid, wherein the surface tension of said first liquid is reduced.--

--9 (amended). The method of claim 8 wherein said first liquid is contained in a vessel and said vessel is in a sonic bath comprising a second liquid.

-- 10 (amended). The method of claim 8 wherein beads are present in said first liquid.

--11 (amended). The method of claim 8 wherein said first liquid is at an alkaline pH.

12. The method of claim 9 wherein the temperature of said second liquid is about 65°C to about 75°C.

13. The method of claim 12 wherein the cells are mycobacterial cells.

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